American Journal of Human Genetics, Volume *88*

Supplemental Data

Mutations in Frizzled 6 Cause Isolated Autosomal-Recessive Nail Dysplasia

Anne-Sophie Fröjmark, Jens Schuster, Maria Sobol, Miriam Entesarian, Michaela BC Kilander, Dana Gabrikova, Sadia Nawaz, Shahid M. Baig, Gunnar Schulte, Joakim Klar, and Niklas Dahl

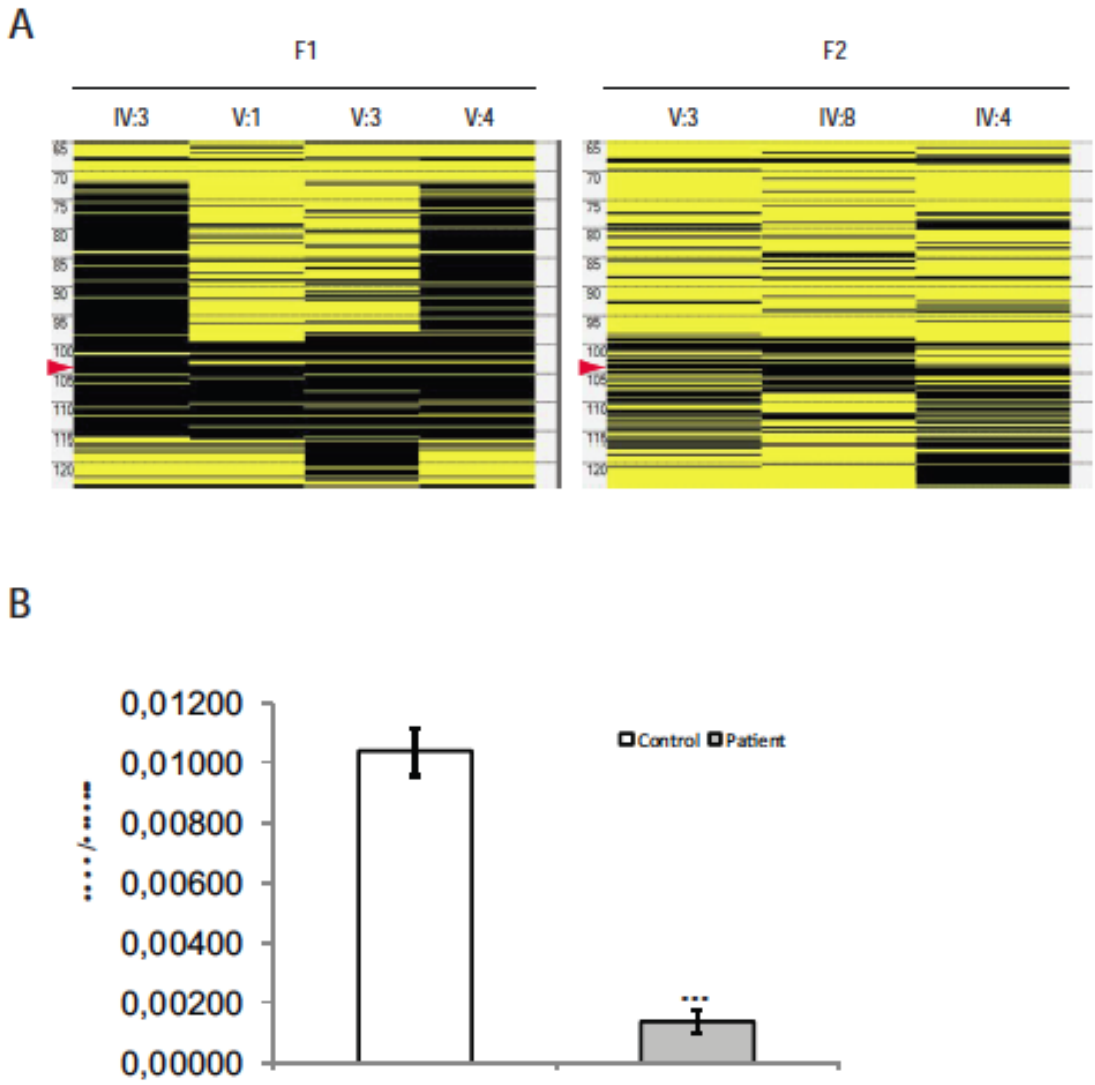

Figure S1. Genome-Wide SNP Array Analysis and Quantification of *FZD6* mRNA on Primary Fibroblast Cultures

(**A**) Autozygosity mapping from part of chromosome 8q on four individuals from family F1 and three individuals from family F2. The arrows denote the position of *FZD6*. SNP genotyping was performed with samples from four affected individuals in F1 and three affected individuals in F2 using the GeneChip Human Mapping 250K SNP Array and Assay kit (Affymetrix) according to manufacturer's instructions. Array image data was acquired and analyzed with Affymetrix GeneChip Operating Software (GCOS) 1.4. SNP allele calling was done with Affymetrix GeneChip Genotyping Analysis Software (GTYPE) 4.1. Genotype data were generated as tab delimited text files and imported into the computer program AutoSNPa[15].

(**B**) Quantification of *FZD6* mRNA on primary fibroblast cultures derived from a control individual and the affected individual V:1 (F1). Total RNA was prepared

using TRIzol® reagent (Invitrogen) from primary fibroblast cultures derived from a healthy Pakistani control individual and individual V:1 of family F1. cDNA was synthesized from 1µg RNA using a RevertAid^{TM} H Minus First Strand cDNA Synthesis Kit (Fermentas) according to manufacturer's protocols. qPCR of *FZD6* transcript levels was normalized to β-actin. Primer sequences are available upon request. Error bars denote means ± standard deviation (SD).

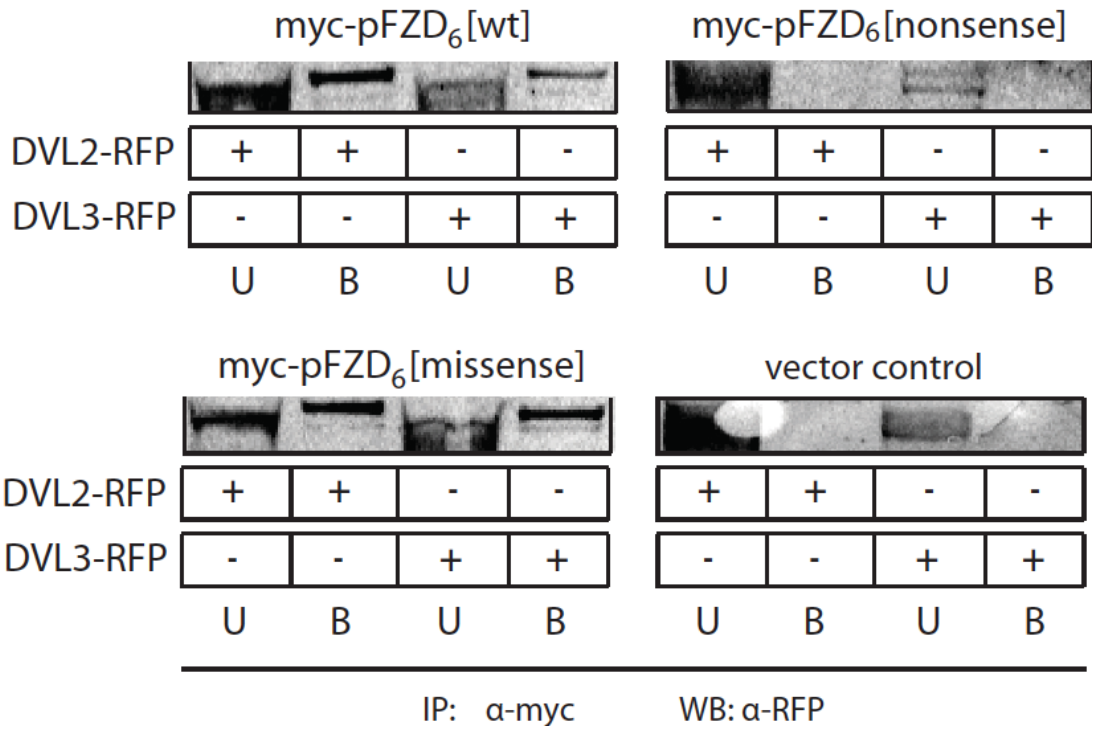

Figure S2. Interaction of FZD_6[wt] and FZD_6[missense] and Dishevelled 2 and Dishevelled 3

Interaction of FZD_6[wt] and FZD_6[missense] and Dishevelled 2 and Dishevelled 3 (DVL2, DVL3) respectively, visualized after co-immunoprecipitation. U denotes unbound fractions and B denotes bound fractions. FZD_6[nonsense] did not show any co-immunoprecipitation. HEK293T cells were cultured in RPMI 1640 medium (Sigma). Growing cells at a confluence ~80% in 10 cm dishes were transfected with either 8 µg myc-p*FZD6*[wt], myc-p*FZD6*[nonsense] or myc-p*FZD6*[missense] or an empty vector control in combination with 5 µg p*DVL2*-RFP or p*DVL3*-RFP, respectively, for co-immunoprecipitation. After two days, cells were checked for RFP expression by fluorescence microscopy on an LSM510 confocal microscope (Zeiss) and harvested using a cell scraper and centrifuged for 5 min at 280 x g. To study FZD_6-DVL interaction, we used a Dynabeads® Protein G Immunoprecipitation kit (Invitrogen) according to the manufacturer's protocol. Proteins were immunoprecipitated with an α-myc-antibody (Santa Cruz Biotechnology). Protein fractions were separated on a 10% NuPage Bis/Tris Gel (Invitrogen) and subsequently transferred to a PDVF membrane using the iBlot transfer system (Invitrogen). Co-immunoprecipitated DVL proteins were detected with an α-RFP antibody (Clontech) and proteins were visualized and quantified using the Odyssey infrared imaging

system and software (LI-COR Bioscience). The coding sequences for human Dishevelled 2 and 3 (DVL2, DVL3) were amplified from clones IOH9688 and IOH21682 (imaGenes) and inserted into pDsRed-Monomer-Hyg-N1 (Clontech), respectively.

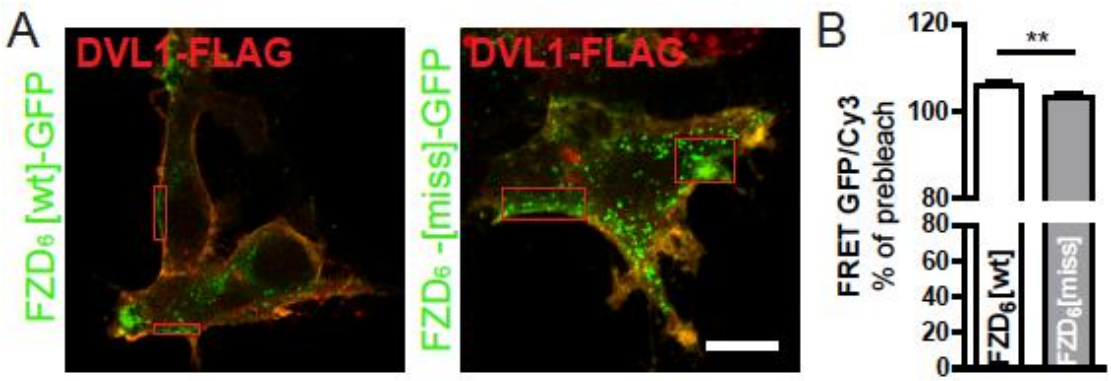

Figure S3. FRET Analysis

FRET analysis of GFP and Cy3 after photoacceptor bleaching of 28 randomly chosen HEK293 cells expressing either FZD_6[wt]-GFP or FZD_6[missense]-GFP together with DVL1-FLAG. (**A**) Representative examples of cells analyzed with FRET. Red squares indicate areas of photobleaching/FRET measurements. (**B**) Diagram showing that the association between FZD_6[wt]-GFP and DVL1-FLAG is closer than that between FZD_6[missense]-GFP and DVL1-FLAG (n=28 for each receptor). Error bars denote ± standard deviation of the mean (SEM). Size bar: 10 µm.